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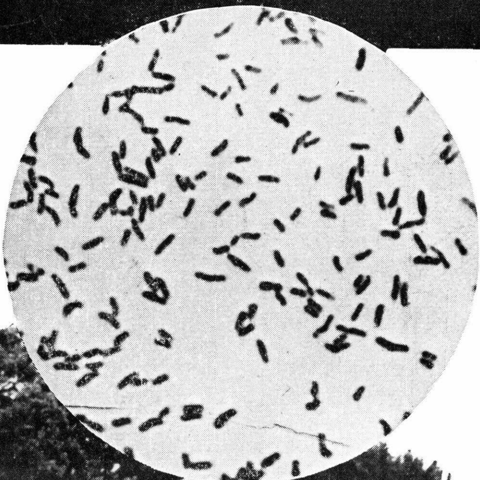
LEGUME INOCULATION



What it is
What it does

Farmer's Bulletin No. 2003
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LEGUMINOUS plants—used for hay, seed, or cover crops or for pasture—are valuable in increasing crop production and in improving soil quality. They accomplish this desirable double purpose, in combination with the work of associated soil bacteria, by taking nitrogen from the air and fixing it in the soil. The ability of legumes to do this can be increased manyfold *if we inoculate the seed* with legume bacteria before planting.

What is inoculation? How is it done? What are its effects? These and other questions about legume bacteria are answered in this bulletin. It supersedes Farmers' Bulletin No. 1784, Nitrogen-Fixing Bacteria and Legumes.

Washington, D. C.   Issued December, 1948

COVER-PAGE ILLUSTRATION shows field tests of different soybean bacteria. Middle rows were inoculated with poor culture; rows at either side with good cultures. Upper inset, legume bacteria, highly magnified.

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Legume Inoculation: What It Is; What It Does

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Contents

INOCULATION of legumes means the introduction of legume bacteria into the soil for the purpose of increasing the production of legume crops and improving the soil.

The actual increase in legume-crop growth due to inoculation of the seed is dependent upon the effectiveness of the legume bacteria used and also upon soil and climatic conditions. Effective legume inoculants have contributed both directly and indirectly to solving some of the world's food problems.

Well-inoculated legumes contain nodules (small lumps) produced by effective legume bacteria. All three—the legume, the nodule, and the bacteria—are necessary for the nitrogen-fixation process. The nitrogen fixed may vary from 50 to 100 pounds or more per acre per year, depending on the plant, the soil, and the climatic conditions.

Proper inoculation may mean the difference between success and failure of the legume crop, especially on new legumes growing for the first time in a soil.

The relative importance of leguminous plants for feeding, for soil improvement, and for soil conservation depends primarily upon the effectiveness of the nitrogen-fixing bacteria living in the nodules on the plant roots.

Legume Inoculation, an Important Discovery

The beneficial association of soil bacteria with legumes was discovered in 1886. This discovery proved beyond doubt that with the help of these bacteria legumes can use the nitrogen from the air as well as from the soil and in addition can enrich the soil in which they grow. It had such practical results that, almost immediately, numerous laboratory and field studies were begun, both in this country and in Europe, to learn more about the activities of these micro-organisms, now known to most agriculturists as legume bacteria.

In 1901 the United States Department of Agriculture began its investigations on methods of inoculating legumes. Since then, through a number of its publications, the Department has given farmers accurate knowledge on how to increase crop yields by this practice and also has pointed out to scientific workers the precautions they must take to get satisfactory results. Many State agricultural experiment stations also have issued bulletins, circulars, and scientific papers, and several have published books on the subject of legume bacteria.

As our knowledge of legume-bacteria secrets increases we encounter increasing numbers of legume-inoculation problems. This is especially true in our changing soil and climatic conditions, and particularly as we introduce new species or varieties of legumes. Even now the subject of improvement in legume inoculation provides the stimulus for much-needed research work. The few scientists in the laboratory, the greenhouse, and the field engaged in this work have not as yet gathered sufficient information on which to base recommendations for the beneficial inoculation of all legume crops.

The Legumes

Legumes are beanlike, in that they bear their seeds in pods. They belong to the family that botanists call the Leguminosae. Of the more than 10,000 known species, only about 200 are cultivated by man, and in the United States only about 50 are grown commercially. These species are further divided into varieties. For instance, more than 100 named varieties of soybeans are being grown in this country. The best known and most widely used legumes are the following:

Alfalfas	Persian clover	Beans
Sweetclovers	Sub clover	Winter peas
Bur-clovers	Soybeans	Black locust
Black medic	Peas	Crotalarias
Red clover	Vetches	Sesbania
White clover	Cowpeas	Alyceclover
Crimson clover	Lespedezas	Peanuts
Hop clover	Lupines	Trefoils

The legumes are rich in high-quality protein and in calcium, are fairly well supplied with phosphorus, and are a good source of vitamins, especially A and D. These qualities make legumes rate as one of man's best foods and as almost indispensable for efficient, economical livestock feeding.

The protein in legumes is directly related to high nitrogen content, and in this respect they differ markedly from grasses and other nonlegumes. The average protein content of 1 ton of eight legume hays is compared with the protein in eight grasses in table 1.

These figures show that legume hays are about twice as rich in protein as the grasses. Their protein content will vary with the stage of maturity. For example, before bloom, alfalfa has about 380 pounds of protein per ton, and red clover about 375 pounds, as compared with the averages 295 and 235 pounds, respectively, given in table 1.

TABLE 1.—*Protein content of legume and of nonlegume hays*

Legume	Average protein content per ton	Nonlegume	Average protein content per ton
	Pounds		*Pounds*
Vetch (hairy)	390	Bromegrass	200
Cowpea	370	Bluegrass	165
Field pea	300	Oat hay	165
Alfalfa	295	Corn fodder	155
Soybean	295	Orchard grass	155
White clover	290	Redtop grass	145
Lespedeza	255	Rye hay	135
Red clover	235	Timothy	125
Average	304	Average	156

Legumes have gained great popularity and economic importance wherever they have been grown. This is because (1) they have high nitrogen content and feeding value, (2) they contain a large proportion of readily decomposable organic matter, and (3) they are versatile in fitting into special farm practices, especially soil conservation.

Nitrogen and Root Nodules

The air we breathe is primarily a mixture of nitrogen and oxygen gases. About 80 percent by volume is pure nitrogen in a free or uncombined state. Above every acre of land surface there is about 35,000 tons of this free nitrogen, which, as such, is totally useless to plant or animal life. Nitrogen has been called the aristocrat of all the elements—it is stubbornly opposed to entering into combination with other elements. Under powerful influences, however—such as lightning discharges and chemical reactions brought about by tremendous heat—the air nitrogen does combine to form compounds that are used to supply industrial and agricultural needs.

Fortunately, for obtaining atmospheric nitrogen for farming operations, nature has provided farmers with a simpler and less expensive method—the growing of inoculated legumes. Soon after the legumes begin to grow, the legume bacteria invade the tiny root hairs and multiply in large numbers, forming growths called nodules. A definite partnership is established—one representing a true symbiosis, or a living together of two organisms to the advantage of both—the legume plant furnishing the necessary sugar or energy and the bacteria using this energy material to fix the free nitrogen of the atmosphere. This is called symbiotic nitrogen fixation.

Just how these bacteria do this work is still unknown. Apparently, however, they work with remarkable ease, because controlled experiments have given evidence of nitrogen fixation after 2 or 3 weeks. A deeper, darker green in inoculated legumes than in those uninoculated is one sure sign of nitrogen fixation by the bacteria. In fact, color differences are more reliable for judging nitrogen fixation than numbers of nodules.

Inoculated legumes growing in normal soils display definite characteristic types of nodule formation. Some of these types are shown in figures 1, 2, and 3.

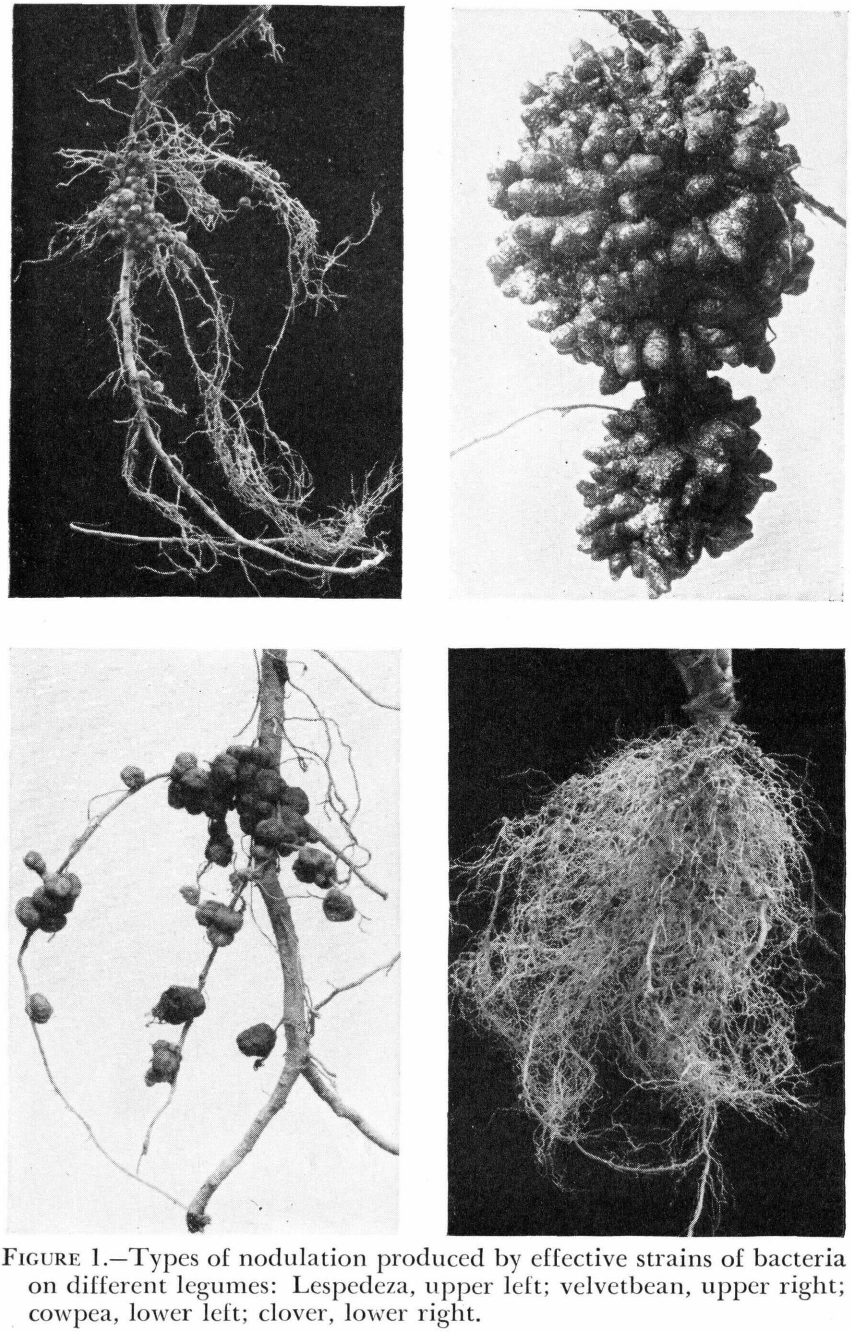

FIGURE 1.—Types of nodulation produced by effective strains of bacteria on different legumes: Lespedeza, upper left; velvetbean, upper right; cowpea, lower left; clover, lower right.

The clustering of nodules around the taproot at the point where the inoculated seed is planted generally indicates that they were formed by the bacteria added in the inoculant. Nodules scattered over the side roots are usually formed by the legume bacteria naturally present in the soil.

In examining legumes to note the effects of seed inoculation it is always well to dig plants at different stages of growth. Nodules come and go with varying moisture levels in the soil. Three examinations should ordinarily be made—the first about 3 or 4 weeks after planting, the second during the middle of the growing period (if moisture conditions are favorable), and the third when the legume is in full blossom. As the legumes mature,

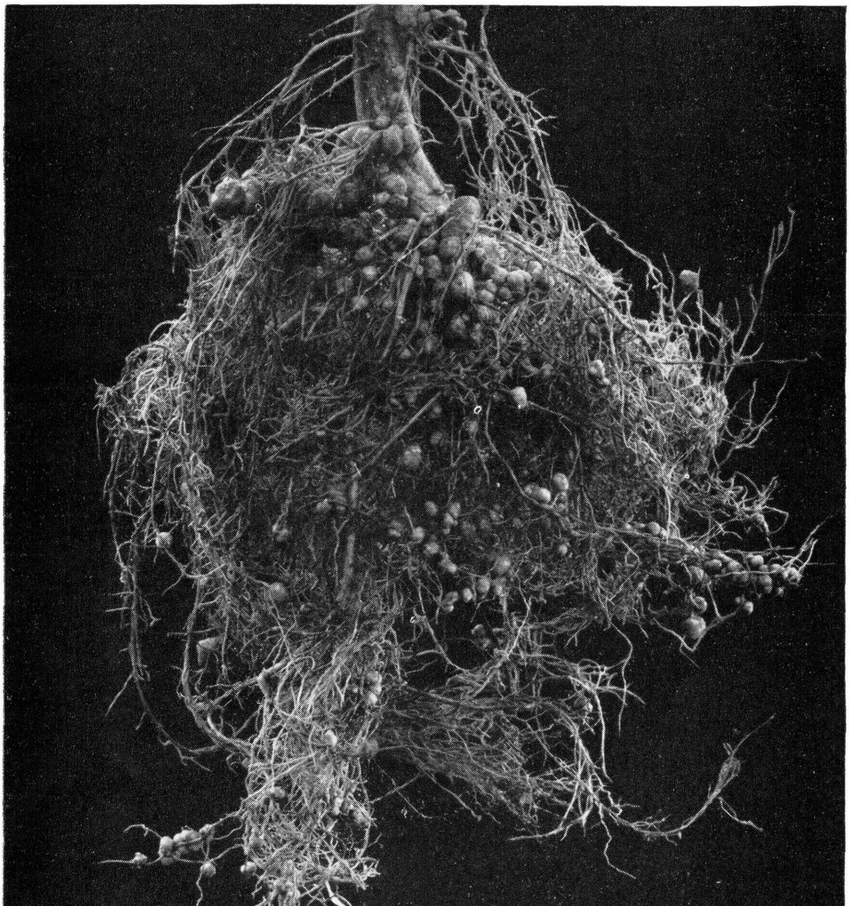

FIGURE 2.—Highly effective nodulation on soybean—clusters around taproot produced by inoculant added to seed; nodules on side roots formed by soybean bacteria in the soil.

the reserve food stored in the nodules is moved to the seeds and on some legumes the nodules begin to disintegrate rather rapidly.

Nitrogen Fixation by Legumes

The quantity of nitrogen taken from the air and fixed by the legume bacteria for different legumes is difficult to calculate. It varies with (1) the kind of legume, (2) the effectiveness of the legume bacteria, (3) the soil conditions, and (4) the presence of

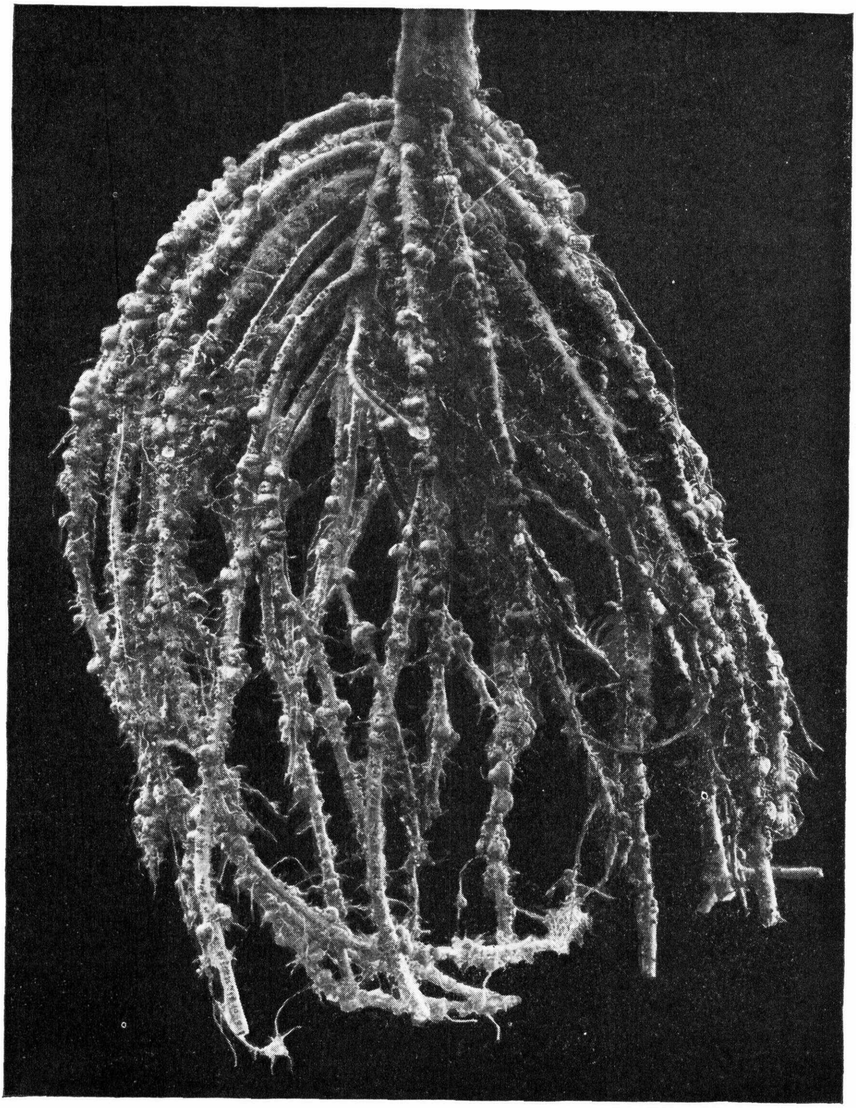

FIGURE 3.—Typical roots of a peanut plant, showing an abundance of nodules.

necessary plant-food elements exclusive of nitrogen. In soils well supplied with available nitrate nitrogen there may be little or no fixation, as the legume plants seem to use this available nitrogen rather than encourage the bacteria to fix more of it.

Because of these many factors and our lack of an accurate method for determining fixation under field conditions we have meager information on the relative quantities of nitrogen fixed by inoculated legumes. Available estimates have been calculated in most cases from controlled pot experiments, which, when magnified to the acre basis, give results higher than the actual figures.

The average number of pounds of nitrogen fixed per acre by some of the important legumes, based on numerous pot experiments and on a few recorded field experiments, is given in table 2.

TABLE 2.—*Average fixation of nitrogen per acre by legumes*

Legume crop	Average fixation of nitrogen per acre	Legume crop	Average fixation of nitrogen per acre
	Pounds		*Pounds*
Canning peas	132	Red clover	89
Mixed legumes	122	Cowpeas	86
Sweetclover	117	Hairy vetch	79
Alfalfa	100	Soybeans	63

The Legume Bacteria

Legume bacteria are single-celled micro-organisms varying in size and shape with age and with the composition of the medium in which they grow. Under the ordinary microscope with a magnification of 1,000 diameters they may be either the usual rod forms, 0.5 to 0.9 micron wide and 1.2 to 3 microns long (a micron is 1/25,000 inch), or the irregular X and Y, or club-shaped, forms shown in figure 4.

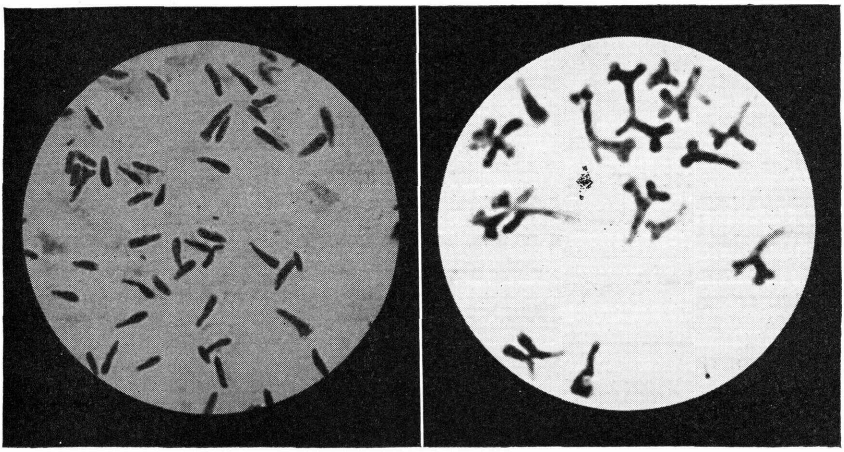

FIGURE 4.—Appearance of legume bacteria through a powerful microscope: Left, Clover bacteria; right, irregular X and Y shapes.

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When young, legume bacteria are extremely motile; they have been observed to have either one polar flagellum (propeller organ) or a number of flagella surrounding the cell. The soil bacteriologist has studied their physiological properties and can distinguish them from other bacteria.

That legume bacteria are of different kinds has been known for a long time. For example, the bacteria that work on alfalfa and sweetclover will not function on the clovers or on peas, beans, soybeans, and other legumes. Conversely, the clover organisms fail to work on alfalfa and sweetclover.

The fact that the legume bacteria are so definitely selective was responsible for the recognition of so-called cross-inoculation groups of leguminous plants. Any plant within such a group can be inoculated with a culture of the proper bacteria, usually comprising several strains known to inoculate effectively all the legumes in that particular group.

Cross-inoculation groups, however, have been open to criticism from the scientific viewpoint because some strains of bacteria are effective for more than one group. But for all practical purposes these groups offer a convenient and workable plan for the preparation of inoculant cultures. Farmers have been accustomed to ordering legume cultures according to group designations, such as the alfalfa group, the clover group, the pea and vetch group, and others. Seven of these groups are now recognized. They are given below, with a list of the legumes in each.

ALFALFA GROUP

Common name	Scientific name	Common name	Scientific name
Alfalfa	*Medicago sativa*	Tifton bur-clover	*M. rigidula*
Buttonclover	*M. orbicularis*	Yellow alfalfa	*M. falcata*
California bur-clover	*M. denticulata*	White sweetclover	*Melilotus alba*
Spotted bur-clover	*M. arabica*	Hubam sweetclover	*M. alba annua*
Black medic	*M. lupulina*	Yellow sweetclover	*M. officinalis*
Snail bur-clover	*M. scutellata*	Bitterclover (sour-clover)	*M. indica*
Tubercle bur-clover	*M. tuberculata*	Fenugreek	*Trigonella foenumgraecum*
Little bur-clover	*M. mimina*		

CLOVER GROUP

Alsike clover	*Trifolium hybridum*	Zigzag clover	*T. medium*
Crimson clover	*T. incarnatum*	Ball clover	*T. nigrescens*
Field hop clover	*T. agrarium*	Seaside clover	*T. willdenovii*
Small hop clover	*T. dubium*	Lappa clover	*T. lappaceum*
Rabbitfoot clover	*T. arvense*	(No common name)	*T. michelianum*
Red clover	*T. pratense*	Puff clover	*T. fucatum*
White clover	*T. repens*	Large hop clover	*T. procumbens*
Sub clover	*T. subterraneum*	Persian clover	*T. resupinatum*
Strawberry clover	*T. fragiferum*	Carolina clover	*T. carolinianum*
Berseem clover	*T. alexandrinum*	Rose clover	*T. hirtum*
Cluster clover	*T. glomeratum*	Buffalo clover	*T. reflexum*
		Hungarian clover	*T. pannonicum*

SOYBEAN GROUP

All varieties of soybeans	*Glycine max* (*Soja max*)

Pea and Vetch Group

Common name	Scientific name	Common name	Scientific name
Field pea	*Pisum arvense*	Purple vetch	*V. atropurpurea*
Garden pea	*P. sativum*	Monantha vetch	*V. articulata*
Austrian Winter pea	*P. sativum* (var. *arvense*)	Sweet pea	*Lathyrus odoratus*
Common vetch	*Vicia sativa*	Rough pea	*L. hirsutus*
Hairy vetch	*V. villosa*	Tangier pea	*L. tingitanus*
Horsebean	*V. faba*	Flat pea	*L. sylvestris*
Narrowleaf vetch	*V. angustifolia*	Lentil	*Lens culinaris* (*esculenta*)

Cowpea Group

Cowpea	*Vigna sinensis*	Pigeonpea	*Cajanus cajan* (*indicus*)
Asparagus-bean	*V. sesquipedalis*	Guar	*Cyamopsis tetragonoloba*
Common lespedeza	*Lespedeza striata*	Jackbean	*Canavalia ensiformis*
Korean lespedeza	*L. stipulacea*	Peanut	*Arachis hypogaea*
Sericea lespedeza	*L. cuneata*	Velvetbean	*Stizolobium deeringianum*
Slender bushclover	*L. virginica*	Lima bean	*Phaseolus lunatus* (*macrocarpus*)
Striped crotalaria	*Crotalaria mucronata*	Adzuki bean	*P. angularis*
Sunn crotalaria	*C. juncea*	Mung bean	*P. aureus*
Winged crotalaria	*C. sagittalis*	Tepary bean	*P. acutifolius* var. *latifolius*
Mat bean	*Phaseolus aconitifolius*	Partridge-pea	*Chamaecrista fasciculata*
Florida beggarweed	*Desmodium tortuosum*	Acacia	*Acacia linifolia*
Tick trefoil	*D. illinoense*	Kangaroo-thorn	*A. armata*
Hoary tickclover	*D. canescens*	Wild-indigo	*Baptisia tinctoria*
Kudzu	*Pueraria thunbergiana*	Hairy indigo	*Indigofura hirsuta*
Alyceclover	*Alysicarpus vaginalis*		
(No common name)	*Erythrina indica*		

Bean Group

Garden beans, kidney bean, Navy bean, pinto bean	*Phaseolus vulgaris*	Scarlet Runner bean	*P. coccineus* (*multiflorus*)

Lupine Group

Blue lupine	*Lupinus angustifolius*	Sundial	*L. perennis*
Yellow lupine	*L. luteus*	(No common name)	*L. diffusus*
White lupine	*L. albus*	(No common name)	*L. villosus*
Washington lupine	*L. polyphyllus*	Serradella	*Ornithopus sativus*

In addition to these seven groups the following legumes appear to require specific strains of legume bacteria for effective inoculation:

Specific Strain Group

Birdsfoot trefoil	*Lotus corniculatus*	Hemp sesbania	*Sesbania exaltata*
Big trefoil	*L. uliginosus*	Sanfoin	*Onobrychis vulgaris* (*sativus*)
Foxtail dalea	*Dalea alopecuroides*	Crown vetch	*Coronilla varia*
Black locust	*Robinia pseudoacacia*	Siberian pea-shrub	*Caragana arborescens*
Trailing wild bean	*Strophostyles helvola*	Garbanzo	*Cicer arietinum*
		Leadplant	*Amorpha canescens*

This grouping of legumes is of great practical value, for it is obviously not necessary to have a specific culture of legume bacteria for every legume to be planted. It is necessary and extremely important, however, to have a sufficient number of different strains of known effectiveness in an inoculant to inoculate properly all the legumes specified on the culture label.

Strain Variation Among Legume Bacteria

That not all legume bacteria are the same has been repeatedly emphasized. Some prefer certain specific groups of legumes, others only a single species. Another difference is the variation in effectiveness between strains of bacteria isolated from the same legumes and from different legumes within the same group.

This type of strain variation among the legume bacteria has great practical significance. Different strains vary widely in their effectiveness when tested on legumes grown under controlled conditions. As nitrogen fixers, some are high, some are poor, and others show gradations between these extremes.

The method for studying strain variation has also shown the existence of parasitic strains of legume bacteria. These parasitic, or ineffective, strains enter the root and form numerous small nodules, but fail to fix any nitrogen or otherwise benefit the plant (fig. 5).

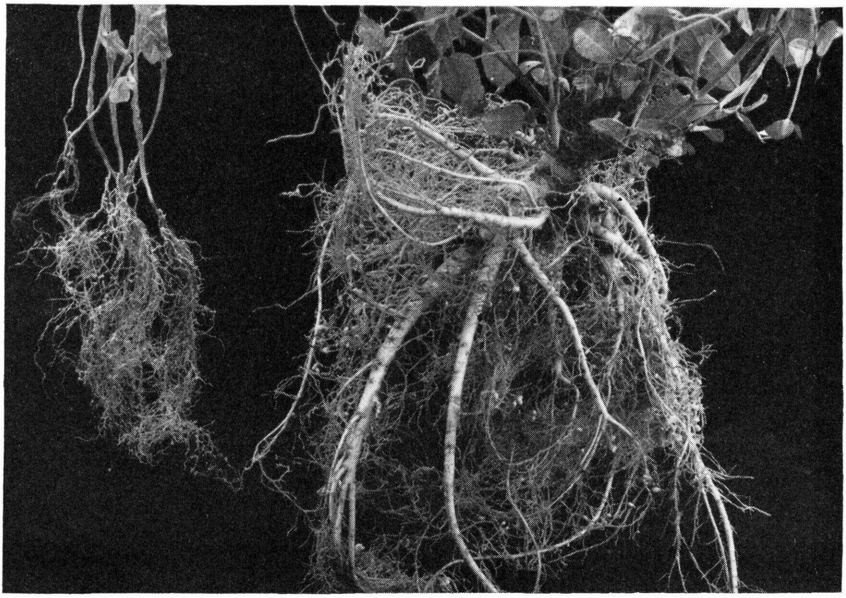

FIGURE 5.—Parasitic, or ineffective, vs. effective inoculation on sweetclover. Parasitic strain of bacteria invaded plants on the left, formed numerous small nodules, but fixed no nitrogen. The large plant on the right was inoculated with a high nitrogen-fixing strain.

This discovery of parasitic strains has made the number of nodules of less importance as a measure of value of a legume inoculant. Numbers of nodules fail to tell the whole story of the effectiveness of the bacteria in fixing nitrogen. It is necessary to measure plant-growth responses, particularly mass, vigor, color, and, if possible, total nitrogen content. Producers of commercial cultures today realize the great importance of using only highly effective nitrogen-fixing strains of bacteria in the preparation of their legume inoculants. The search for new and better strains is continuous, for the cultures that prove of greatest benefit under field conditions will be the ones in greatest demand by farmers. Strain variation among legume bacteria is illustrated in figures 6 to 9.

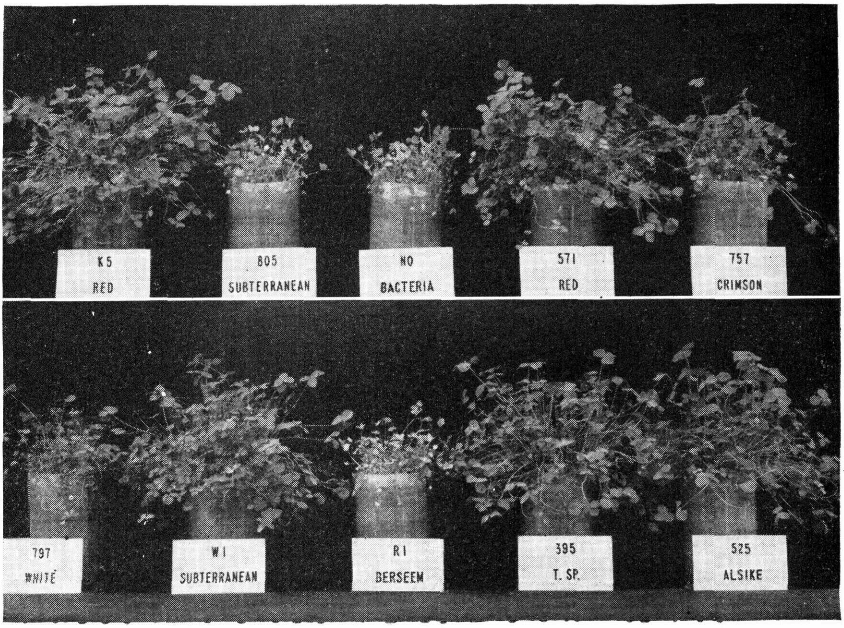

FIGURE 6.—Effect of different strains of clover bacteria on Ladino clover. (Strain R1, isolated from berseem clover, proved to be highly effective on all clovers tested except red and white.)

Some interesting and important observations should be emphasized.

1. The alfalfa and sweetclover strains will work on alfalfa or sweetclover equally well, but they fail to produce nitrogen fixation in bur-clovers and fenugreek. Strains from bur-clover and fenugreek, on the other hand, will work and fix nitrogen on bur-clovers, fenugreek, alfalfa, and sweetclovers.

2. Strains from red and white clovers fix nitrogen on their host plants, but not all of them will effectively inoculate crimson

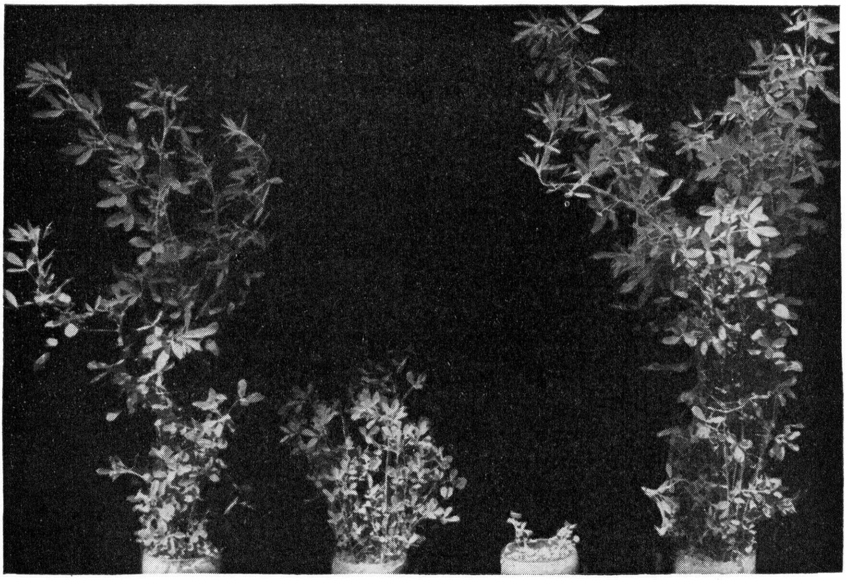

FIGURE 7.—Effect of four strains of alfalfa-sweetclover bacteria on alfalfa. The culture used in the third pot from the left was definitely parasitic.

FIGURE 8.—Effect of four strains of clover bacteria on crimson clover. The poor strain used in the container on the left was effective on both red and white clover.

clover. One strain isolated from berseem clover was effective on all clovers tested except the white and the red.

3. Strains of legume bacteria show definite varietal preferences. For example, some soybean bacteria work on one or two soybean varieties better than on others. The same is true for different varieties of canning or freezing peas.

4. Strains of legume bacteria from birdsfoot trefoil may be highly effective on its host but totally ineffective on big trefoil,

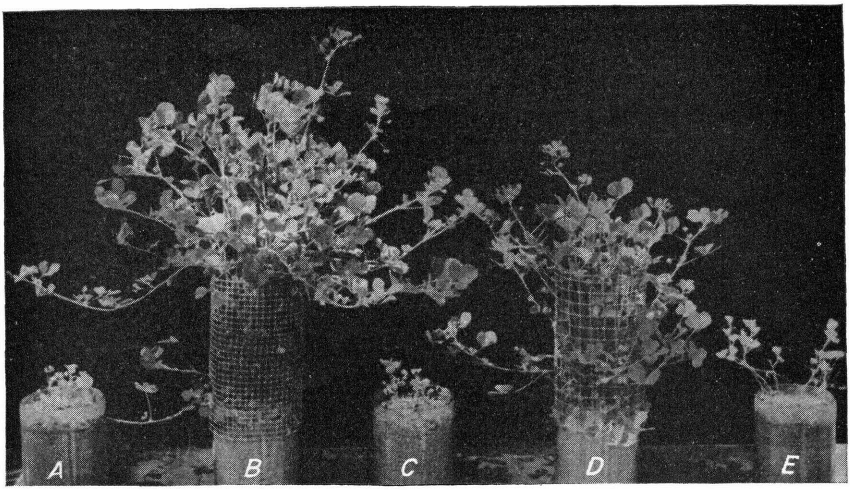

FIGURE 9.—Effect of five strains of legume bacteria on bur-clover. The bacteria used were isolated from (*A*) black medic, (*B*) bur-clover, (*C*) alfalfa, (*D*) fenugreek, and (E) sweetclover.

which is another species. Strains that may be highly effective on big trefoil fail to work on birdsfoot trefoil.

Strain-variation studies have opened the way for soil bacteriologists and producers of commercial inoculants to prepare more effective cultures for all kinds of legumes. In many instances specific strains have been shown to be more effective for specific legumes. Preparation of such cultures has been particularly desirable for experimental plantings of new legumes in new areas.

Necessity for Inoculation

Not all agricultural soils contain the bacteria necessary to promote successful growth in legumes. In some cultivated soils, the legume bacteria naturally present are of the mediocre, or relatively ineffective, type of nitrogen fixers. For example, 100 soybean fields in Wisconsin were examined and nodules collected for isolating the soybean bacteria. When these strains were tested on soybeans it was found that 25 percent were highly effective, 50 percent only average, and the rest poor or ineffective. Even though the legume bacteria for a certain legume may be present in a given soil, the questions always arise, Are they there in sufficient numbers? and, Are they the high-nitrogen-fixing strains?

Farmers cannot be sure before they plant a legume that sufficient bacteria of the proper kind are present in their soils. Nor can they be sure that the bacteria in the nodules on a previous legume were of maximum benefit to that crop. It is entirely possible that the bacteria left in the soil may lose their beneficial properties, that is, their ability to fix appreciable quantities of air nitrogen.

Too often it is taken for granted that inoculation is not necessary, because a legume was grown in the same soil. If the proper bacteria are not present the young legume plants look spindly, sickly, or yellow and may or may not show nodules on the roots. Such cases present a real problem, because it is so much more difficult to get growing plants inoculated than to inoculate the seeds before planting. The necessity for inoculating crimson clover is illustrated in figure 10.

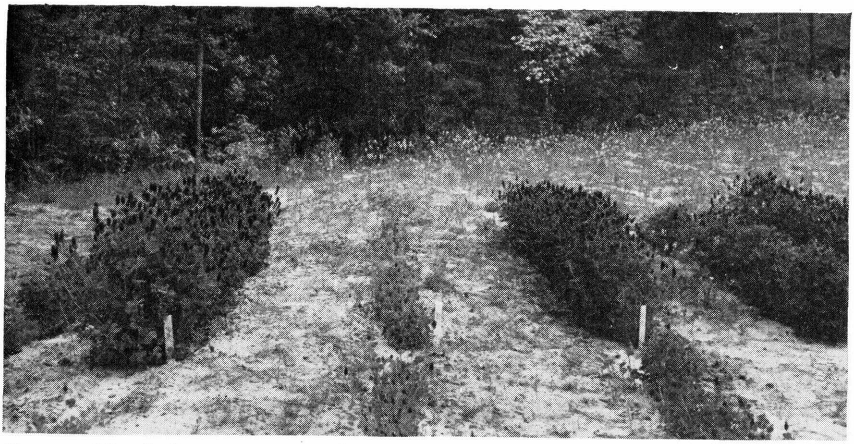

FIGURE 10.—It is necessary to inoculate crimson clover. Where no inoculation was used, a total failure of crimson clover followed. Where the seeds were effectively inoculated excellent growth was obtained.

The one fundamental purpose of legume inoculation is to add a fresh culture of effective strains of legume bacteria to the seed (preferably), so that when the young plant begins to grow, the bacteria will be right there to enter the tiny root hairs and begin their beneficial work in the early stages of the plant's growth.

Now that farmers can purchase legume inoculants prepared with the most effective strains, the simplest, easiest, and most economical way to insure successful growth is to inoculate legume seeds before each planting. Good sound advice to all farmers is, ***Inoculate in all cases of doubt and always on new land.***

Conditions that Affect Legume Bacteria

In the natural competition between inhabitants of the soil, the legume bacteria have the advantage that they are protected from time to time in nodules. Without this association, they would have adverse soil conditions to cope with. Conditions essential to the satisfactory growth of legumes must be fulfilled before maximum results with inoculation can be expected. The principal requirements are the proper preparation of the soil; the presence of an available supply of lime, phosphorus, and potash; and the use of healthy adapted viable seed.

Once nodule bacteria are in the soil, they are subject to the conditions existing there; if the chemical reaction of the soil is

suitable and sufficient moisture and plant food are present, the bacteria should function normally. Acid soils tend to eliminate the bacteria to the degree that these organisms are not tolerant to acid conditions. Nodule bacteria of alfalfa, sweetclover, and red clover are among those that are not very acid-tolerant. Soybean, velvetbean, cowpea, vetch, lespedeza, and lupine bacteria belong to the acid-tolerant types.

Cultures of nodule bacteria are alive and should be treated as living things. They will tolerate low much better than high temperatures. Exposing them to heat that is unbearable by man, or even uncomfortable, may impair or destroy their effectiveness. Inoculating material should therefore be stored in a cool place until used.

Although legume bacteria will tolerate sunlight to some degree, avoid unnecessary exposures either of the unopened containers or of seed that has been treated. Bacteria that dry on seed soon die. If inoculated seed must be kept as long as 48 hours it is advisable to reinoculate. For this reason the purchase of preinoculated seed is not advisable.

Seed treated with legume bacteria should not come in direct contact with caustic lime or mixed fertilizers. Inoculated seed may be drilled down the same spout with superphosphate or basic slag without injury to the bacteria. If the concentration of fertilizer does not injure seed germination it will not ordinarily harm legume bacteria.

Most seed disinfectants are toxic to legume bacteria. Consequently, legume seed that has been treated with disinfectant compounds should not be inoculated in the usual manner. In large-scale operations, the inoculum is mixed with wheat middlings, sawdust, or other inert material and drilled in advance of planting. This practice, called "preplanting" of the bacteria, is successful in areas where it becomes necessary to treat the seed.

Preparation and Use of Commercial Cultures

Soon after the discovery that legume bacteria can fix nitrogen in nodules on the plant roots, soil bacteriologists began to cultivate these organisms on artificial media in the laboratory. A few years later, prepared cultures were offered for sale to farmers. These early cultures were made with specific bacteria for a specific kind of legume, and under field conditions their use resulted in varying degrees of success.

The few failures did not discourage the originators of commercial cultures. Greater success came as they developed more suitable media and improved methods of application. Gradually a farmer demand was created for commercial inoculants, and a number of companies began to manufacture legume cultures and to sell them mostly through seed dealers.

To prepare effective cultures for legumes, the persons engaged in the work must have specialized training and experience. Adequate laboratory facilities, equipment for controlled production, and greenhouse space or other suitable means for testing plants are prerequisites to satisfactory production.

Routine work in such a laboratory calls for periodic tests and transfers of all strains of legume bacteria used in the production of commercial inoculants. These tests are made for purity and for effectiveness on the growing plants. The isolation of new strains is an important feature of the work. These must be purified and tested also. A selection is made of the most effective strains, and a given number are used for the production of the different culture groups. For example, 5 or 6 strains may be used for a culture to inoculate the alfalfa group, whereas 10 to 12 strains may be used in the production of a soybean inoculant.

The bacteria are grown either in liquids or on the surface of agar. Heavy suspensions of bacteria are used for mixing with the carrier, which may be a finely ground peat, or mixtures of peat and charcoal, peat and sand, or other materials. The three types of carriers generally used are (1) moist humus or finely ground peat, (2) agar, and (3) liquid. The great bulk of commercial inoculants are prepared in moist humus. Most seed suppliers handle one or more brands of commercial legume inoculants and thus make them readily available to the consumer trade. Because of the perishable nature of these living bacteria, dealers are cautioned not to store the cultures in places that are either too warm or too dry.

Be sure that the culture is prepared for the specific seed you wish to plant, and use it before the expiration date.

In using agar cultures, add a small quantity of clean cool water to the bottle, shake it vigorously to get the bacteria in suspension, add more water, and then pour the bacterial suspension on the legume seeds and mix them until all are moistened.

In using peat or humus-type inoculants, either (1) moisten the seeds with water and empty the contents of the container on them, mixing until all are coated with the black substance; or (2) add a specified quantity of water to the inoculant to form a thin paste and pour it on the seeds.

Specific directions for using commercial cultures are found on the label of each container.

A thorough mixing is essential in all cases; do not use too much water; avoid soaking the seeds. The results of a field test of different soybean cultures are shown in figure 11.

Plant the seeds as soon as possible after they are inoculated with legume bacteria cultures. Ideal conditions prevail shortly before a gentle rain. When inoculated small legume seeds have remained on or near the surface of the soil exposed to hot drying winds for several weeks, supplemental inoculation is advisable. This may be done by mixing a legume inoculant with cottonseed meal, wheat middlings, or even sand and broadcasting the mixture over the soil when moisture conditions are favorable.

When young legume plants show lack of proper inoculation it may be desirable to reseed the area with inoculated seeds. The bacteria added on the seed may eventually gain access to the root hairs of the growing plants and produce successful inoculation.

FIGURE 11.—A field test of different soybean cultures. At the left the dark-green growth was brought about by effective nitrogen-fixing soybean bacteria. In the right foreground soybeans inoculated with several unsuited cultures could not be distinguished from the uninoculated yellowish-green plants.

The demand for effective legume inoculants has shown a rapid and steady increase since World War I. Estimates gathered from producers and agricultural colleges indicated that in 1929 about 1,500,000 bushel-size units were distributed in the United States. A survey of the industry in 1940 indicated an increase to about 11,135,000. A survey for the year 1946 showed that about 17,536,000 units were prepared for distribution to farmers and large growers of various legumes. The total retail value of the inoculants bought by farmers in 1940 was about $3,000,000 and in 1946 about $4,000,000.

These figures show that the legume-inoculant industry has grown to such proportions as to play an important role in American agriculture. Probably no commodity purchased by farmers gives greater returns per dollar invested. The average cost (1948) for soybean inoculants when purchased in units of large size is about 10 cents per bushel; the cost of the 1-bushel-size unit for alfalfas and clovers is about 50 cents. This size will inoculate 3 to 5 acres, depending upon the rate of seeding. Seed inoculation is cheap crop insurance for legumes.

Inspection of Legume Inoculants

To protect farmers from buying worthless cultures, certain control agencies have been set up. In 1916 the United States Department of Agriculture began testing commercial legume inoculants in accordance with an act of Congress providing for soil microbiological investigations. Cultures have been procured each year in the open market and tested on the legume plants for which prepared. From time to time results of these tests have been published along with the names of the manufacturers or distrib-

utors. This testing work is still carried on by the Department, although to a more limited extent than in the past. The testing process consists of inoculating seed according to directions on the container and planting them in sterile sand moistened with a sterile nutrient solution. Great care is taken to prevent entry of nodule bacteria and the transfer of bacteria from one seed pot to another. A culture is considered unsatisfactory if under these conditions it fails to produce nodules, to increase plant growth, or to produce a plant that is darker green than uninoculated controls.

In addition to testing commercial legume inoculants over this period, the Department has kept in close touch with inoculant producers. Its representatives have visited the more important laboratories and offered to help the manufacturers in production problems and thus assure better cultures for the farmer. A few of the States also have control agencies to protect their farmers from unscrupulous producers and dealers. All these agencies have had a decidedly beneficial effect in bringing about improvements and raising the standard and quality of inoculants. The results obtained with two good cultures on Austrian Winter peas are shown in figure 12.

Commercial cultures for legumes have reached a high state of reliability and can be very generally depended upon to produce satisfactory results. In a recent test, only 3 of a total of 133 cul-

FIGURE 12.—Austrian Winter peas respond to inoculation. Two good cultures were used on the rows to the left, and the typical nodulation produced is shown in the inset. Where no inoculation was used (on right) the plants failed to make satisfactory growth.

tures for alfalfa were unsatisfactory, 8 of 122 for clovers, 5 of 138 for soybeans, and 9 of 98 for peas and vetch; all of 47 tested for cowpeas were satisfactory, and all of 61 for lespedeza were good. These tests of the most important legumes show that farmers are getting legume inoculants of good quality.

Research with Legume Bacteria

Despite the progress that has been made with legume inoculation, a number of practical problems as well as many of a scientific nature still await solution. The response of hairy vetch to good inoculation is shown in figure 13.

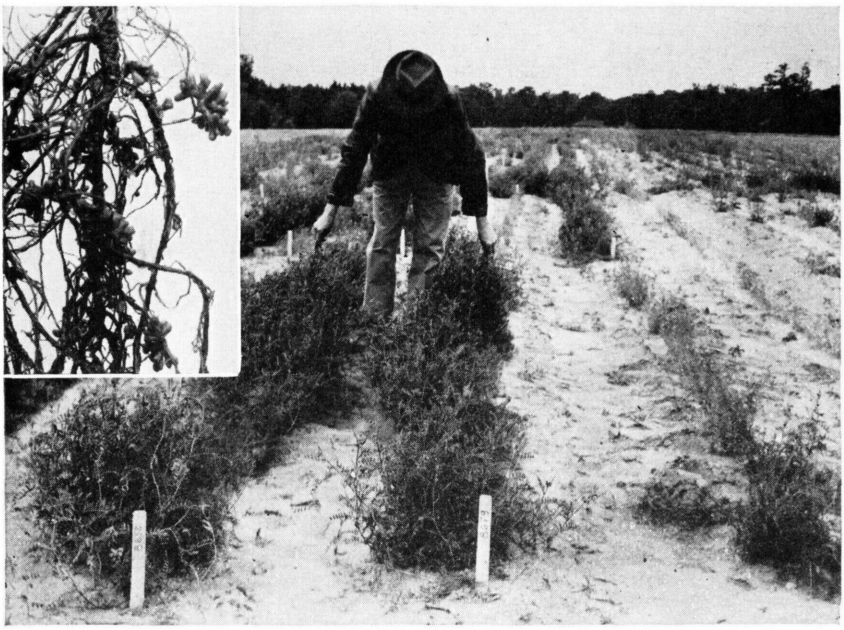

FIGURE 13.—Effect of proper inoculation of hairy vetch, one of the best winter cover crops for certain sections of the South. The uninoculated row on the right was a total failure. Typical vetch nodulation is shown in the inset.

In the Department's research on the improvement of legume inoculation, one phase has been concerned with obtaining and making available for experimental use a collection of highly effective strains of legume bacteria. Studies have been made of the physical, chemical, and biological factors that influence the efficiency, activity, and longevity of these bacteria for different legumes growing under widely varying soil and climatic conditions. A number of workers in the different experiment stations have also been studying the physiology of these micro-organisms and the mechanism of nitrogen fixation.

Some of the companies preparing commercial inoculants maintain research departments, and all are searching for strains of legume bacteria superior to those naturally present in the soil. Large acreages of lima beans, navy beans, kidney beans, and peanuts are planted each year without inoculation, because it has not been proved that these legumes respond to inoculation as do other legumes. Reasons for these apparent abnormalities are being sought.

The use of chemical seed disinfectants on certain legumes has raised obstacles to the program of more successful inoculation. One or more of these seed treatments were found to be compatible with legume bacteria to a certain degree, but workers are searching for ways to inoculate legume seeds successfully and treat them chemically without having to drill separately.

Economic Importance of Legume Inoculation

The effective inoculation of legumes has been a controlling factor in increasing the yield and quality of farm crops. It makes available greater quantities of the high-protein feeds so necessary in livestock production. Legumes add much nitrogen to the soil and in addition they contribute greatly to the maintenance of its organic-matter content. Organic matter improves the physical properties of soils, increases their moisture-holding capacity, and absorbs plant-food elements. The activity of soil organic matter determines its real value. If the organic matter is high in nitrogen and the soil is well supplied with calcium, other soil microorganisms break it down. With necessary additions of fertilizer it then provides a readily available supply of plant food for crop production.

By increasing the nitrogen content of the soil through greater growth of legumes and organic matter, the legume bacteria, therefore, play three important roles in the world's food program: (1) They increase the supply of high-protein feeds for domestic animals; (2) they increase the yield of soybeans, peas, and other legumes valuable to man for food; and (3) indirectly, they increase the production of other farm crops.

In the United States, of the potential area of about 498,300,000 acres that can be put under cultivation, approximately one-fifth is planted to some legume crop—hay, seed, cover, or pasture. Such recognition of the value of leguminous plants is well founded and shows the great importance of legumes in agriculture. Carrying out a successful inoculation program, along with expanding the legume acreage, will make agriculture even more profitable.

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